ORIGINAL RESEARCH

REFEREED

Adverse Effects of Zilpaterol Administration in Horses: Three Cases

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ABSTRACT

Three healthy horses were fed the beta-adrenergic agonist feed additive zilpaterol at a dosage of 0.17 mg/kg body weight to study zilpaterol elimination kinetics. Soon after ingestion of zilpaterol, the horses developed skeletal muscle tremors and tachycardia. A 75 to 87.5% reduced dose of zilpaterol was fed to the horses 24 hours after the initial dose; administration was discontinued thereafter. The horses exhibited restlessness, muscle tremors, and profuse sweating 20 to 25 minutes after ingestion of zilpaterol. Tachycardia developed within 40 minutes and took up to 2 weeks to resolve. Muscle tremors lasted up to 1 week. The most pronounced derangements in serum biochemistry were increased activities of lactic dehydrogenase, creatine kinase, and aspartate transferase, indicating muscle damage. The most severely affected horse also had transient azotemia, hematuria, and proteinuria, suggesting renal damage. All three horses recovered without treatment and were clinically normal 2 to 3 weeks after the initial dose of zilpaterol. Because of their anabolic properties, beta-adrenergic feed additives are considered a risk for abuse in performance horses, despite the absence of Food and Drug Administration approval for such use. Oral administration of zilpaterol to horses at the dosage indicated for use in cattle may result in prolonged adverse effects, including tachycardia, muscle tremors, and renal damage.

Keywords: Horses; Zilpaterol; Adverse effects; Beta-agonists; Tachycardia

INTRODUCTION

Zilpaterol is a beta-adrenergic agonist feed additive approved by the Food and Drug Administration (FDA) in the United States for feeding to cattle to improve weight gain and enhance carcass leanness. Other beta-adrenergic agonist drugs include ractopamine and clenbuterol. Clenbuterol is the only beta-adrenergic agonist drug approved in the United States for administration to horses, for use in the management of airway obstruction. Because of their muscle-building properties, there is a risk that drugs in this class will be used illegally in performance horses. Consequently, regulatory agencies require data on the disposition of such drugs so that appropriate screening regimens for performance horses may be designed.

To gain more information about the elimination of zilpaterol in the urine of horses, the drug was fed to the three horses described herein. The adverse effects observed, though they were consistent with those expected for beta-adrenergic agonists in horses, were of unexpected severity and duration. Veterinarians and others in the equine industry should be made aware of the risk of prolonged adverse effects when zilpaterol is administered to horses.

CASE REPORT

Three registered Quarter Horses were fed zilpaterol (Zilmax, Intervet Inc, Millsboro, DE) mixed with grain as part of a prospective study designed to evaluate urinary zilpaterol depletion. Horse 1 was a 469-kg, 4-year-old gelding, horse 2 was a 479-kg, 3-year-old filly, and horse 3 was a 462-kg, 5-year-old mare. All three horses had tested negative for the hyperkalemic periodic paralysis gene. The horses were privately owned; the owner had provided informed consent for their use in the study, which was conducted at the Animal Metabolism-Agricultural Chemicals Research Unit (AMACRU) as part of a Native American Internship Program sponsored by the United States Department of Agriculture (USDA-ARS). The study protocol was approved by the Animal Care and Use Committee at the USDA-ARS AMACRU, and the horses were under the supervision of a veterinarian at all times.

After 7 days of acclimation to the research facility and collection of urine and heart rate baseline data, the horses were fed 0.17 mg/kg body weight zilpaterol in 0.5 pounds of grain mix. All horses completely consumed the grain–zilpaterol mixture within 5 minutes of feeding. Within 20 to 25 minutes of being fed the drug, all three horses began to sweat. The horses became agitated and

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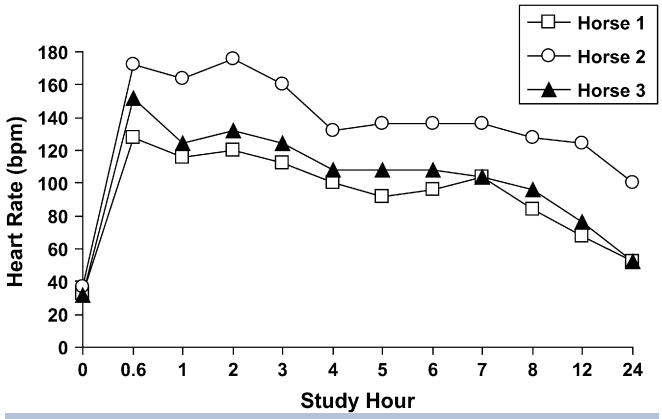

Figure 1. Heart rates in three horses for 24 hours after consumption of 0.17 mg/kg zilpaterol.

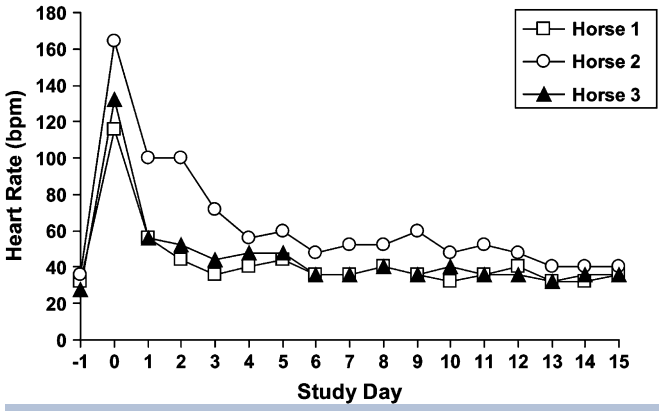

Figure 2. Heart rates in 3 horses for 15 days after consumption of 2 daily doses of zilpaterol.

began to lick and mouth their pens. Horses 1 and 2 became flatulent, and horse 2 exhibited pawing behavior.

All three horses developed markedly increased heart rates within 40 minutes after drug consumption. Heart rates for the three horses during the first 24 hours and for 15 days after initial zilpaterol consumption are shown in Figures 1 and 2. Horse 1, which had a mean baseline heart rate of 33 beats per minute (bpm), had a heart rate of 128 bpm within 40 minutes after feeding, a 387% increase over baseline. Within the same period, heart rates for horse 2 and horse 3 increased from baselines of 37 and 32 bpm to 172 bpm (464% of baseline) and 152 bpm (475% of baseline), respectively. The horses began to sweat profusely, particularly across their necks, shoulders, backs, and flanks. Within 90 minutes of consuming zilpaterol, the horses had developed muscular tremors, which began in the skeletal muscles of the neck, shoulder, and foreleg and spread throughout the visible skeletal muscles. Intermittent visible muscular tremors continued for up to 1 week after the initial dose of zilpaterol.

Twenty-four hours after the initial feeding of zilpaterol, all horses remained tachycardic; horses 1 and 3 had heart rates of 52 bpm, whereas horse 2 had a heart rate of 100 bpm. The adverse effects observed after the first dose were expected to be transient based on the investigators' previous experience with beta-adrenergic agonists in farm animal species and literature reports of the acute effects of other beta-adrenergic agonists in horses. Zilpaterol feeding was therefore continued on the second day of the trial, but at a reduced dosage of 0.043 mg/kg, one fourth the initial dose. Horse 2 did not consume all of the feed that was offered; the dose consumed on the second day by horse 2 was 0.022 mg/kg. Heart rates were not as markedly increased after the second feeding of zilpaterol. Horse 1 peaked at 62 bpm approximately 90 minutes after the second dose, horse 2's heart rate remained at 100 bpm after the second dose, and the heart rate of horse 3 increased from a pre-feeding level of 52 bpm to a peak of 72 bpm approximately 180 minutes after feeding of the second dose.

One hour after feeding the second dose, blood was drawn from a jugular vein of each horse into a plain glass tube and submitted to the Veterinary Diagnostic Laboratory at North Dakota State University for serum chemistry analysis (VetTest 8008 and VetLyte, IDEXX Laboratories, Westbrook, ME). Horse 1 had mildly elevated activity of aspartate transaminase (AST) and creatine kinase (CK) and mild hypoalbuminemia. Horse 2 had marked elevations in AST, CK, and lactic dehydrogenase (LDH) activity, and glucose, blood urea nitrogen (BUN), and creatinine levels, a mild elevation in alkaline phosphatase (ALP) activity, and mild hyponatremia and hypochloremia. Horse 3 had markedly increased AST, CK, and LDH activity, elevated BUN, creatinine, and glucose, and mild hyponatremia and hypochloremia. Serum chemistry measurements were repeated at intervals over the 2 weeks after initial drug administration; results are summarized in Table 1. Because of the unexpected persistence of adverse effects in these horses, the initial study protocol of 7 consecutive days of drug administration was altered, and drug administration was discontinued after the second dose.

Serum chemistry measurements were repeated in all horses 2, 3, and 8 days after the initial dose of zilpaterol. AST activity decreased over time for all horses, and they all had hypoalbuminemia on days 2, 3 and 8. Horse 2 had a more significantly increased BUN and creatinine than horses 1 and 3, which persisted longer; these values had returned to normal in all horses by 8 days after treatment. Other abnormalities seen in one or more horses on repeat serologic evaluations were elevated ALP and LDH activity, elevated calcium, glucose, and potassium concentrations, and lowered sodium and chloride levels. In horse 1, heart rate returned to baseline levels for the first time 3 days after the initial dose (Fig. 2), and of the 10 observations made on the 4th day, half were at or below baseline

Table 1. Serologic abnormalities observed in three horses after consumption of zilpaterol[a]

Days Post-treatment	1 Day			2 days			3 Days			8 Days			14 Days
Horse Number	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**2**
Test (Normal Range[b])													
Albumin (3.8–4.7 mg/dL)	***2.8***	3.9	3.9	***2.9***	***3.5***	***2.9***	***3***	***3.1***	***2.9***	***2.8***	***2.8***	***2.7***	***2.8***
Alkaline phosphatase (10–469 U/L)	170	***542***	285	153	***528***	231	140	409	256	114	255	206	254
Aspartate transferase (0–317 U/L)	***395***	***657***	***1,324***	***384***	***1,101***	***1,842***	***363***	***>1,083***	***1,886***	295	***921***	***1,308***	***626***
Blood urea nitrogen (11–22 mg/dL)	23	***37***	***29***	***23***	***57***	***27***	22	***60***	17	21	20	19	20
Calcium (9.9–12.4 mg/dL)	12.5	10.8	12.1	***12.5***	10.3	11.6	***13***	10.2	10.9	***12.9***	***12.9***	***12.5***	***12.5***
Creatine kinase (1–354 U/L)	503	***3,132***	[c]	171	***5,142***	***1,551***	135	***4,285***	***653***	116	***533***	***540***	211
Creatinine (0.4–1.8 mg/dL)	1.7	***4.2***	***2.7***	1.5	***6.8***	1.8	1.6	***4***	1.5	1.5	1.4	1.3	1.5
Gamma-glutamyl transpeptidase (0–50 U/L)	18	39	37	20	46	32	18	39	36	16	34	31	32
Globulin (2.4–4.0 g/dL)	4	4	3.7	3.5	***4.3***	3.8	3.4	3.7	***4.2***	3.1	3.5	3.6	3.4
Glucose (58–167 mg/dL)	95	***>686***	***351***	93	150	130	91	89	98	90	90	84	6.9
Lactate dehydrogenase (0–1337 U/L)	1,162	***1,946***	***2,948***	793	***>2,800***	***3,217***	830	***>2,800***	***2,585***	664	***2,382***	***1,803***	***1,550***
Total bilirubin (0.0–2.5 mg/dL)	1.1	1.2	2.3	1	1.8	1.6	1	1.7	1.9	0.8	0.8	0.9	0.5
Total protein (5.2–8.5 g/dL)	6.8	7.8	7.6	6.4	7.8	6.7	6.4	6.8	7.1	5.9	6.3	6.3	6.2
Sodium (132–146 mmol/L)	134	***118***	***123***	135	***128***	***127***	134	133	***128***	138	142	138	141
Potassium (2.4–4.7 mmol/L)	4.4	2.9	3.1	***5.1***	2.5	3.7	***4.8***	<1.5	3.4	4.3	4.2	3.6	4.3
Chloride (97–108 mmol/L)	98	***78***	***83***	105	***85***	***87***	104	***86***	***92***	104	106	104	106

[a] Italicized values are outside normal ranges.
[b] Reference ranges provided by IDEXX Laboratories, Westbrook, ME for IDEXX VetTest 8008.
[c] Equipment malfunction: value not available.

level. The heart rate of horse 3 was not more than 12.5% above baseline for half of the observations from day 6 to day 12 after treatment began and returned to baseline on day 13 after the beginning of treatment.

Horse 2, the 3-year-old filly, had the most pronounced and long-lasting clinical signs and serum biochemical abnormalities. Horse 1 did not display a depressed attitude or appetite at any time, and Horse 3 had only a mildly depressed attitude and appetite the day after the first dose. Horse 2, in contrast, had depression and decreased appetite the first day after treatment began, depression on day 2, and decreased appetite on days 2 and 3 after treatment. All observations of heart rate in horse 2 remained at 48 bpm or more, an increase of 30% over baseline, through day 12 after treatment began; heart rate did not drop to baseline level until 16 days after treatment. Because horse 2 had pronounced and prolonged clinical signs and the most serious biochemical derangements, additional diagnostic procedures were performed. Automated complete blood count (CBC) (QBC Autoreader, IDEXX Laboratories, Westbrook, ME) 3 and 4 days after treatment indicated mild leukocytosis and granulocytosis 3 days after the initial treatment and a mild decrease in packed cell volume 4 days after treatment. Eight days after treatment, automated CBC again showed marginally increased platelet count, with rare acanthocytes and neutrophils with toxic changes observed microscopically. Examples of neutrophils with toxic changes to the cytoplasm (basophilia, granularity, and vacuolization), from horse 2, are shown in Figure 3. Automated CBC 14 days after treatment revealed marginally increased white blood cell and platelet counts; on microscopic examination some acanthocytes and neutrophils with toxic changes were observed. Hematologic values for horse 2 are summarized in Table 2. Because all changes were mild, they were considered diagnostically insignificant.

Because of concerns about very high levels of BUN and creatinine in the serum of horse 2, urinalysis was performed on days 2, 3, and 4 post-treatment. On day 2, the urine was dark yellow to brown and had a specific gravity of 1.025, with a pH of 5.0 and high levels of protein, bilirubin, and blood or other heme-like molecules present. On day 3, the urine was yellow and the specific gravity had dropped to 1.016, the pH had increased to 6.0, and high levels of blood or heme-like molecules were still present. By day 4 the urine was light yellow, there were only trace amounts of blood, hemoglobin, or other heme-carrying molecules, and the specific gravity was low at 1.008.

All horses recovered from this adverse drug event without treatment and were clinically normal within 2 to 3 weeks.

DISCUSSION

Zilpaterol is a beta-adrenergic agonist feed additive that was approved by the FDA for use in cattle in the United States in 2006. Label indications are increased rate of weight gain, improved feed efficiency, and increased carcass leanness in feedlot cattle. In adipose tissue, beta-adrenergic agonists increase blood flow, increase lipolysis, and decrease lipogenesis, whereas in muscle tissue they increase blood flow and protein accretion.[1] Zilpaterol is the second beta-adrenergic repartitioning agent to be approved by the FDA for use in food animals; ractopamine hydrochloride was approved for use in swine in 1999 and for use in cattle in 2003. In the United States, these are the only beta-adrenergic agents approved for use in food animals, and neither drug is approved for use in horses. Another potent beta-adrenergic agonist, clenbuterol, is approved for the management of airway obstruction caused by bronchospasm or mucus accumulation in the airways of horses. The World Anti-Doping Agency considers beta-adrenergic agonist drugs a risk for abuse because of their muscle-building properties and has classified clenbuterol, zilpaterol, and other beta-adrenergic agonists as prohibited for use in human athletes.[2] There is a risk of abuse in performance animals, too; the beta-adrenergic drug ractopamine has been recovered from the urine of a racehorse in Australia.[3]

Because of the anticipated abuse of this relatively new beta-adrenergic agonist in performance horses, the original purpose of this study was to measure depletion of zilpaterol residues in urine of horses after a short dietary exposure. The original study protocol described a zilpaterol feeding period of 7 days based on previous work.[4] There was no intent to measure side effects of zilpaterol because it was expected that side effects would be minor and of short duration, consistent with previously observed effects in horses treated with beta-adrenergic agonists.[5-7] In a similar study using sheep dosed with 0.15 mg/kg body weight zilpaterol per day, no side effects were noted, although heart rates were not specifically measured.[4] The dose of zilpaterol that was chosen for use in the current study is the label dose for cattle of 0.17 mg/kg body weight. Unlike feedlot cattle, which would receive a total dose of 0.17 mg/kg body weight zilpaterol in the ration throughout the day, and the sheep in the previous study, which had the dose divided between two feedings, horses in this study were provided a bolus dose of zilpaterol formulated into a single 227-g grain supplement. Horses were fed the grain before their daily allotment of hay, so the complete zilpaterol dose was given in a single, rapidly consumed portion. The rapid absorption of a bolus dose equivalent to the dose that is usually delivered over a 24-hour period to cattle likely exacerbated the observed adverse effects.

The most commonly reported side effects associated with beta-adrenergic agonist treatment in most animal species are muscle tremors and increased heart rate.[5,6] In horses, sweating is a side effect that also has been associated with clenbuterol administration.[7] Symptoms normally decrease

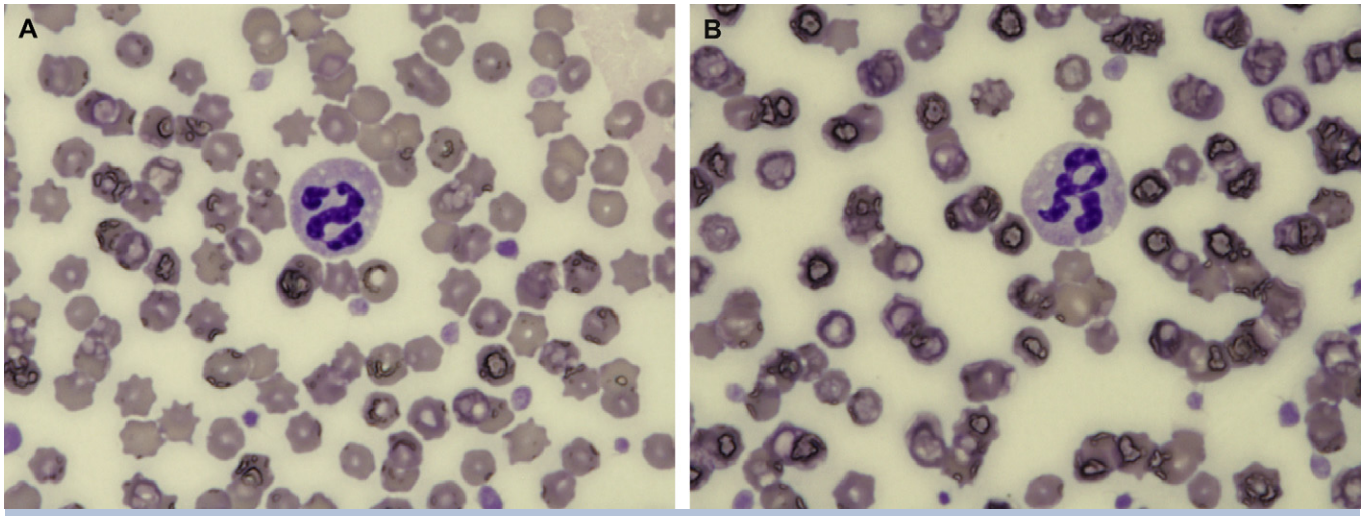

Figure 3. Neutrophils with toxic changes observed in the blood of horse 2 after zilpaterol administration.

Table 2. Complete blood count values for horse 2[a]

	Days Post-treatment			
Test (Normal Range)	**3 Days**	**4 Days**	**8 Days**	**14 Days**
Hematocrit (32.0–52.0%)	40.8	*30.9*	*30.7*	32.7
Hemoglobin (11.0–19.0 g/dL)	15.0	11.3	11.3	12.0
White blood cells ($6.0–12.5 \times 10^9$/L)	*14.6*	10.4	12.5	*13.0*
Granulocytes ($2.8–8.0 \times 10^9$/L)	*9.5*	5.7	7.5	7.2
Lymphocytes + monocytes ($2.1–7.0 \times 10^9$/L)	5.1	4.7	5.0	5.8
Platelets ($90–350 \times 10^9$/L)	*337*	291	*403*	*356*

NOTE. Numbers in italics are outside normal range.
[a] Reference ranges provided by IDEXX Laboratories, Westbrook, ME, for QBC Autoreader.

over time and resolve within a few hours. In the horses that were fed zilpaterol in this case, the clinical signs observed were unsurprising, but the severity and duration of signs were greater than anticipated. The expectation that clinical signs would be transient, as is typically observed, informed the decision to feed the horses a reduced dose of zilpaterol on the second study day. When it became clear that the adverse effects would not be transient as expected, zilpaterol administration was discontinued.

Increased serum activity of LDH, measured in all three horses, and increased serum activity of CK and AST, measured in the two female horses, are consistent with muscle damage. The increased serum activities of these enzymes in the horses fed zilpaterol, combined with the observation of pronounced, prolonged tachycardia and skeletal muscle tremors, are evidence that some muscle cellular damage resulted from direct or indirect effects of the drug. Moderate to severe muscle damage also causes the release of myoglobin from the muscle tissue, which, like hemoglobin, may bind to the tubules of the kidney, causing tubulonephrosis and related effects such as obstructed blood flow through the tubules and restricted blood supply to the kidneys. In severe cases, renal failure may result. The brownish color of the urine, marked proteinuria, and marked increases in serum BUN and creatinine in horse 2 could be signs of myoglobinuria and a transient reduction in renal function. Hemoglobinuria appears less likely, because other parameters for hemolysis were not evident. However, the mild decrease in post-treatment packed cell volume (PCV) in combination with color changes of the dipstick indicating bilirubinuria, hemoglobinuria, and proteinuria prevent entirely ruling out the possibility that slight post-treatment hemolysis took place in horse 2. The two female horses also had markedly increased blood glucose the day after they were first fed zilpaterol (>686 mg/dL in horse 2 and 351 mg/dL in horse 3). This finding suggests that these two horses were more sensitive than the gelding to the direct and indirect effects of the beta-adrenergic agonist drug on glucose metabolism. Stress-induced release of catecholamines may have aggravated the hyperglycemia or reduced glucose utilization in peripheral tissues associated with administration of zilpaterol.[8]

Hypoalbuminemia, which was observed in all three horses, is difficult to explain in the presence of normal total protein level; some degree of renal failure appears a plausible cause. Horse 1 was mildly hyperkalemic, which may be because of muscle damage and cell destruction. Horses 2 and 3, which were more severely affected than horse 1, experienced hyponatremia, most likely caused by sodium loss from profuse sweating or impaired renal sodium transport, and hypochloremia, which may be associated with endogenous glucocorticoid release or renal impairment. Horse 2, which was the most severely affected, also had increased serum alkaline phosphatase activity. Whether this was caused by liver impairment, mucosal damage, bone disorder, glucocorticoid release, hyperglycemia, or even by leukocyte dysfunction cannot be answered. All three horses had mildly increased serum levels of calcium; the explanation for this phenomenon is not known.

All of the horses were fed the same initial dose of zilpaterol, whereas the second dose was one-fourth the initial dose in horses 1 and 3 and one-eighth the initial dose in horse 2, because she did not consume all of her feed. Nevertheless, clinical signs and hematologic abnormalities were mildest in the 4-year-old gelding, more pronounced in the 5-year-old mare, which received the same total dosage as the gelding, and most pronounced in the 3-year-old filly, which received a slightly lower total dose than the other two horses. The severity of adverse effects in these three horses did not correlate with the dosage of zilpaterol they consumed and may be related to differences in body composition, sex, and age.

Although direct comparisons of oral potency between zilpaterol and other beta-agonists, such as ractopamine and clenbuterol, cannot be made for horses, human "no observable effect level" data indicate that clenbuterol is approximately 19 times more potent than zilpaterol, and that zilpaterol is about 125 times more potent than ractopamine.[9] For this reason, it may not be surprising that side effects were not mentioned after bolus administration of either 0.54 or 1.64 mg/kg body weight of ractopamine HCl to horses, doses 3.2 and 9.8 times greater than doses of zilpaterol provided to horses in this study.[10]

Pharmacokinetic data that might be helpful in explaining the side effects observed in this study are not available for zilpaterol. Based on zilpaterol's chemical structure and the fixed steric position of its hydroxyl group (rendering it less likely to be a target for glucuronidation or sulfation relative to a phenol), one might predict that zilpaterol would have a serum half-life greater than ractopamine, but less than clenbuterol.[11]

CONCLUSION

Oral consumption of zilpaterol at the dosage of 0.17 mg/kg body weight produced clinical signs typically associated with beta-adrenergic agonist drug administration, but the duration of the signs and the associated hematologic abnormalities were more pronounced than expected. Because of the potential for extralabel abuse of zilpaterol in performance horses, veterinarians and horse owners should be aware of the possibility that administration of the drug to horses may produce prolonged adverse effects.

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